
Human Recombinant SST5 Somatostatin Receptor Stable Cell Line**Cat. No. M00286****Version 07282020**

I. Introduction	1
II. Background	1
III. Representative Data	2
IV. Thawing and Subculturing	2
V. References	3
VI. Limited Use License Agreement.....	4

I. INTRODUCTION

Catalog Number: M00286

Cell Line Name: CHO-K1/SST5/Gα15

Gene Synonyms: SSTR5, SSTR4, SRIF_{1B}

Expressed gene: Genbank Accession Number NM_001053; no expressed tags

Host cell: CHO-K1/Gα15

Culture Properties: Adherent

Quantity: 2 vial (>1×10⁶ per vial) frozen cells

Stability: More than 16 passages

Application: Functional assay for SST5 receptor

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen), 200 µg/ml Zeocin (Cat. #R250-01, Life Technologies)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

Somatostatin receptors (SSTRs), a family of seven transmembrane (TM) domain G-protein-coupled receptors having five distinct subtypes (termed SSTR1–5), are activated by somatostatin secreted from the nerve and endocrine cells. SSTRs are widely expressed in many tissues, frequently as multiple subtypes that coexist in the same cell. With expressions in a tissue-specific manner, SSTRs are involved in the regulation of secretion of insulin, glucagon and growth hormone as well as cell growth induced by neuronal excitation in both the central and peripheral nervous systems. The five receptors share common signaling pathways such as the inhibition of adenylyl cyclase, activation of phosphotyrosine phosphatase (PTP), and modulation of mitogen-activated protein kinase (MAPK) through G-protein-dependent mechanisms. Aberrant expression of somatostatin receptors is known to be involved in a large number of human tumors.

* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

The human medullary thyroid carcinoma cell line TT expresses all SSTR subtypes. SSTR5 induces cell cycle arrest via PTP-dependent modulation of MAPK, which is associated with the induction of the retinoblastoma tumor suppressor protein and p21.

In addition, SSTR 5 displays acute desensitization of adenylyl cyclase coupling and undergoes rapid agonist-dependent endocytosis.

III. REPRESENTATIVE DATA

Intracellular calcium mobilization assay:

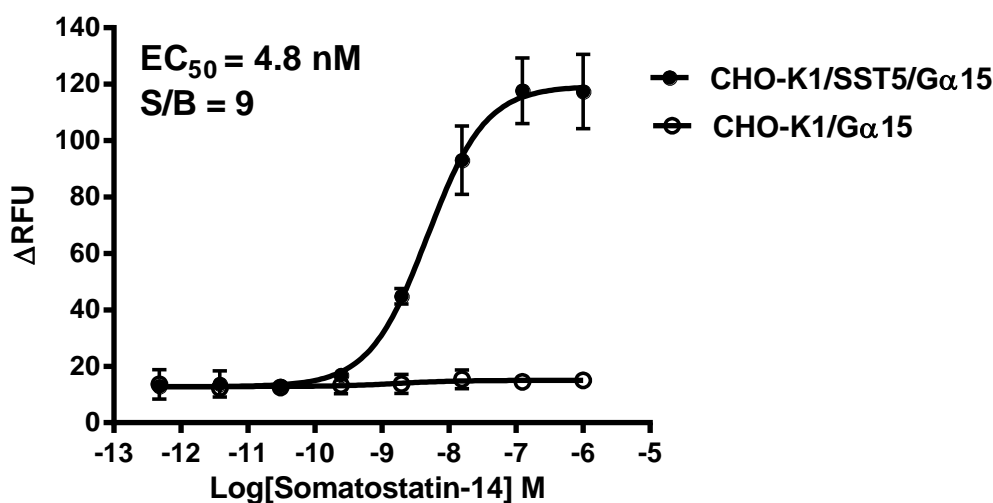


Figure 1. Somatostatin-14-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/SST5/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist somatostatin-14. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (8-fold dilution) of Somatostatin-14 (Mean ± SD, n = 2). The EC₅₀ of Somatostatin-14 on this cell was 4.8 nM.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$
 X is the logarithm of concentration. Y is the response
 Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.

3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO₂.
7. Add antibiotic in the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Yogesh C. Patel (1999) Somatostatin and its receptor family. *Frontiers in Neuroendocrinology* 20(3): 157-198
2. Carruthers A.M., Warner A.J., Michel.A.D., Feniuk W., Humphrey P.P.A. (1999) Activation of adenylate cyclase by human recombinant sst5 receptors expressed in CHO-K1 cells and involvement of Gas proteins. *Br. J. Pharmacol.* 126: 1221-1229
3. Forbes Alderton, Tai-Ping D Fan, and Patrick P A Humphrey (2001) Somatostatin receptor-mediated arachidonic acid mobilization: evidence for partial agonism of synthetic peptides. *Br. J. Pharmacol.* 132(3): 760-766

GenScript USA Inc,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: <http://www.genscript.com>

For Research Use Only.

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

- 1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
- 2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
- 3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 860 Centennial Ave. Piscataway, NJ 08854, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.